

1/18

**FIGURE 1****Blastp vs. NCBI-nr**

>dbj|BAB68513.1| hatching enzyme EHE4 [Anguilla japonica]  
Length = 271

Score = 197 bits (502), Expect = 1e-49  
Identities = 103/233 (44%), Positives = 141/233 (60%), Gaps = 5/233 (2%)

Query: 52 DKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLSATSNNK--WPMGGSGVVEVPFLLS 109  
D D I ++ S L+EGD+I + + +N+ W G+VEVP+ +S  
Sbjct: 41 DPDDVDITTSILQSNNGSSEILMEGDLIVSNTRNAMKCWNNQCLWRKSSDGLVEVPYTVS 100

Query: 110 SKYDEPSHQVILEALAEFERSTCIRFVTTYQDQRDFISIIPMYGCFSSVGRSGGMQVVSLA 169  
+++ + I A+ F TCIRFV QRDFISI GC+S +GR+GG QVVSLA  
Sbjct: 101 NEFSYYHKKRIENAMKTFNTETCIRFVPRSSQRDFISIESRDGCYSYLGRTGGKQVVSLA 160

Query: 170 PT-CLQKGRGIVLHELMHVLGFWHEHTRADRDYIRVNWNEILPGFEINFIKSQSSNMLT 228  
C+ GI+ HEL H LGF+HEHTR+DRD Y+++NW + P NF ++N+ T  
Sbjct: 161 RYGCYV--HGIIQHELNHALGFYHEHTRSDRDEYVKINWENVAPHTIYNFQTQDTNNLNT 218

Query: 229 PYDYSSVMHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGC 281  
PYDY+S+MHYGR AFS G+ TITP+ P+ IGQR ++S DI R+ KLY C  
Sbjct: 219 PYDYTSIMHYGRTAFSTNGMDTITPVPNPNQSIGQRRSMSGDILRIKKLYSC 271

**Tblastn vs. NCBI-est**

Tissue - Uterus tumour

>gb|BI061462.1|BI061462 IL3-UT0117-070301-494-H12 UT0117 Homo sapiens cDNA.  
Length = 652

Score = 175 bits (443), Expect = 2e-42  
Identities = 85/86 (98%), Positives = 85/86 (98%)  
Frame = -2

Query: 29 SCAGACGTSFPDGLTPEGTQASGDKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLS 88  
SCAGACGTSFPDGLTPEGTQASGDKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLS  
Sbjct: 546 SCAGACGTSFPDGLTPEGTQASGDKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLS 367

Query: 89 ATSNKWPMGGSGVVEVPFLLSSKYDE 114  
ATSNKWPMGGSGVVEVPFLLSSKY E  
Sbjct: 366 ATSNKWPMGGSGVVEVPFLLSSKYGE 289

FIGURE 2

Library	Tissue/cell source	Vector	Host strain	Supplier	Cat. no.
1	human fetal brain	Zap II	XL1-Blue MRF <sup>+</sup>	Stratagene	936206
2	human ovary	GT10	LE392	Clontech	HL1098a
3	human pituitary	GT10	LE392	Clontech	HL1097a
4	human placenta	GT11	LE392	Clontech	HL1075b
5	human testis	GT11	LE392	Clontech	HL1010b
6	human substantia nigra	GT10	LE392	in house	
7	human fetal brain	GT10	LE392	in house	
8	human cortex brain	GT10	LE392	in house	
9	human colon	GT10	LE392	Clontech	HL1034a
10	human fetal brain	GT10	LE392	Clontech	HL1065a
11	human fetal lung	GT10	LE392	Clontech	HL1072a
12	human fetal kidney	GT10	LE392	Clontech	HL1071a
13	human fetal liver	GT10	LE392	Clontech	HL1064a
14	human bone marrow	GT10	LE392	Clontech	HL1058a
15	human peripheral blood monocytes	GT10	LE392	Clontech	HL1050a
16	human placenta	GT10	LE392	in house	
17	human SHSYSY	GT10	LE392	in house	
18	human U373 cell line	GT10	LE392	in house	
19	human CFPoc-1 cell line	Uni Zap	XL1-Blue MRF <sup>+</sup>	Stratagene	936206
20	human retina	GT10	LE392	Clontech	HL1132a
21	human urinary bladder	GT10	LE392	in house	
22	human platelets	Uni Zap	XL1-Blue MRF <sup>+</sup>	in house	
23	human neuroblastoma Kan + TS	GT10	LE392	in house	
24	human bronchial smooth muscle	GT10	LE392	in house	
25	human bronchial smooth muscle	GT10	LE392	in house	
26	human Thymus	GT10	LE392	Clontech	HL1127a
27	human spleen 5' stretch	GT11	LE392	Clontech	HL1134b
28	human peripheral blood monocytes	GT10	LE392	Clontech	HL1050a
29	human testis	GT10	LE392	Clontech	HL1065a
30	human fetal brain	GT10	LE392	Clontech	HL1065a
31	human substantia nigra	GT10	LE392	Clontech	HL1093a
32	human placenta #11	GT11	LE392	Clontech	HL1075b
33	human Fetal brain	GT10	LE392	Clontech	custom
34	human placenta #59	GT10	LE392	Clontech	HL5014a
35	human pituitary	GT10	LE392	Clontech	HL1097a
36	human pancreas #63	Uni Zap XR	XL1-Blue MRF <sup>+</sup>	Stratagene	937208
37	human placenta #19	GT11	LE392	Clontech	HL1008
38	human liver 5'stretch	GT11	LE392	Clontech	HL1115b
39	human uterus	Zap-CMV XR	XL1-Blue MRF <sup>+</sup>	Stratagene	980207
40	human kidney large-insert cDNA library	TriplEx2	XL1-Blue	Clontech	HL5507u

1 AGGTCCTTGT GGACAATPAGC TATTCTTCTT GGCTCTGTCTG CTTCCCTTCA CTGGGTGCAG  
61 GTGACTGTGG GGGTGTCCCC AAATGCTGCC CAGCGCTGAC ATGCTCCGCC TCTGGGATTT  
m l r l w d

121 CAATCCAGGT GGGGCCCTGA GTGACCTGGC TCTGGGGCTC AGGGGTATGG AGGAGGGGGG  
f n p g g a l s d l a l g l r g m e e g

181 ATATAGCTGC GCAGGAGCCT GTGGTACCAG CTTCCCAGAT GGCCTCACCC CTGAGGGAAC  
g y s c a g a c g t s f p d g l t p e g

241 CCAGGCCTCC GGGGACAAGG ACATTCTGTC AATTAACCAA GGGCTCATCC TGGAAGAAAC  
t q a s g d k d i p a i n q g l i l e e

301 CCCAGAGAGC AGCTTCCTCA TCGAGGGGGA CATCATCCGG CCGAGTCCCT TCCGACTGCT  
t p e s s f l i e g d i i r p s p f r l

361 GTCAGCAACC AGCAACAAAT GGCCCATGGG TGGTAGTGGT GTCGTGGAGG TCCCTTCTCT  
l s a t s n k w p m g g s g v v e v p f

421 GCTCTCCAGC AAGTACGATG AGCCCAGCCA TCAGGTCATC CTGGAGGCTC TTGCGGAGTT  
l l s s k y d e p s h q v i l e a l a e

481 TGAACGTTCC ACGTGCATCA GGTTTGTAC CTATCAGGAC CAGAGAGACT TCATTTCAT  
f e r s t c i r f v t y q d q r d f i s

541 CATCCCCATG TATGGGTGCT TCTCGAGTGT GGGGCGCAGT GGAGGGATGC AGGTGGTCTC  
i i p m y g c f s s v g r s g g m q v v

601 CCTGGCGCCC ACGTGTCTCC AGAAGGGCCG GGGCATTGTC CTTCATGAGC TCATGCATGT  
s l a p t c l q k g r g i v l h e l m h  
CP1

661 GCTGGGCTTC TGGCACGAGC ACACGCGGGC CGACCGGGAC CGCTATATCC GTGTCAACTG  
v l g f w h e h t r a d r d r y i r v n

721 GAACGAGATC CTGCCAGGCT TTGAAATCAA CTTCATCAAG TCTCAGAGCA GCAACATGCT  
w n e i l p g f e i n f i k s q s s n m

781 GACGCCCTAT GACTACTCCT CTGTGATGCA CTATGGGAGG CTCGCCTTCA GCCGGCGTGG  
l t p y d y s s v m h y g r l a f s r r  
78836-GR1-3'

841 GCTGCCCACC ATCACACCAC TTTGGGCCCC CAGTGTCCAC ATCGGCCAGC GATGGAACCT  
g l p t i t p l w a p s v h i g q r w n

901 GAGTGCCTCG GACATCACCC GGGTCCTCAA ACTCTACGGC TGCAGCCCCAA GTGGCCCCAG  
l s a s d i t r v l k l y g c s p s g p  
78836-GR1nest-3' CP2

961 GCCCCGTGGG AGAGGTGAGT GGCATGGCAG GAAGGTGACT TGAACCTGGA GAAGGCGCCT  
r p r g r g e w h g r k v t -

1021 GTGCTCTAAT GGTGTCAGGG AGGGTGACAA GGAGGGAGAT GAGGTTGCAG GGGGAGCAGG  
1081 GTGAGATCAC GGGGGCTTGC CAC

→ →

**FIGURE 4**

Primer	Name	Sequence (5'-3')
CP1	4C5	ACC GCT ATA TCC GTG TCA A
CP2	4C6	GCT GCA GCC GTA GAG TTT
GeneRacer 3'		GCT GTC AAC GAT ACG CTA CGT AAC G
78836-GR1-3'		AGT GTC CAC ATC GGC CAG CGA TGG AA
GeneRacer 3' nested		CGC TAC GTA ACG GCA TGA CAG TG
78836-GR1nest-3'		ATG GAA CCT GAG TGC CTC GGA CAT C
78836-FL-F	4C7	CTG TCA GCA ACC AGC AAC AA
78836-FL-R	9B2	AGC CAC AGG CTT AAT CTT CG
78836-FL2-F	9E6	TCT ACC ATG GAG GGT GTA GG

5/18

FIGURE 5

```

1  ATGGAACCTG AGTGCCTCGG ACATCACCCG GGTCTCAAAA CTCTACGGCT GCAGCCCAAG
   w n l s a s d i t r v l k l y g c s p

61  TGGCCCCAGG CCCCCTGGGA GAGGGTCCCA TGCCCCACAGC ACTGGTAGGA GCCCCGCTCC
   s g p r p r g r g s h a h s t g r s p a

121 GGCCTCCCTA TCTCTGCAGC GGCTTTTGGG GGCAGTGTCTG GCGGAATCCA GGAGCCCCGA
   p a s l s l q r l l e a l s a e s r s p

181 CCCCAGTGGT TCCAGTGCAG GAGGCCAGCC CGTTCCTGCA GGGCCTGGGG AGAGCCCACA
   d p s g s s a g g q p v p a g p g e s p

241 TGGGTGGGAG TCCCCTGCCC TGAAAAAGCT CAGTGCAGAG GCCTCGGCAA GGCAGCCTCA
   h g w e s p a l k k l s a e a s a r q p

301 GACCCTAGCT TCCTCCCCAA GATCAAGGCC TGGAGCAGGT GCCCCCGGTG TTGCTCAGGA
   q t l a s s p r s r p g a g a p g v a q

361 GCAGTCCTGG CTGGCCGGAG TGTCCACCAA GCCCACAGTC CCATCTTCAG AAGCAGGAAT
   e q s w l a g v s t k p t v p s s e a g

421 CCAGCCAGTC CCTGTCCAGG GAAGCCCAGC TCTGCCAGGG GGCTGTGTAC CTAGAAATCA
   i q p v p v q g s p a l p g g c v p r n

481 TTTCAAGGGG ATGTCCGAAG ATTAAGCCTG TGGCTTCTGT CCCAAGTAG GGAGGGCATC
   h f k g m s e d

541 CTCTGCCCAG TGGAGCTGGG TCGTCTACCT CTTGGCTCCT TTGGGCCACA CCACTGTCTT
601 CCAGCCCCAA CCTACCACCC CATCTCAGAG GGCCAGGACT CTTCCCCTGT CTCTCTTCAC
661 TGTGTTCCCC TAAGGGCTCC TAGGGCCAGG GGTTCTTCTA GCTCTGCCAC AGGGGAAGGC
721 AGGCCTGGCT GTGCCTGCTC TTGACTTTTG CCCAGCCCTG GTGGATGCTG GGAATGGGAG
781 GTGACATTCT CCAGGGACAG GTCCTGGAAG GGGTGGGGAA GAGGTAGGTT CCAGCCCCGC
841 AGAACCCCTG AATCCCTCCT GTGCCTGAGG CCCTGCCCCC CAGCATGGAC TAATGGTGTC
901 CCTACCTCTC CCTCAGGGCA GCCCTGTGGC TGGGACCCTG GGAACAGCCT CCCATCCCAC
961 CCAACATGCC CAAGTGTGGG GGAATGTTCT ACAGCAGTGT AGCCTCCAGC CCTTCTCTCC
1021 AGGAGGCTTT GAGAGCCCAA CTTACTCCCC TGCAGAGCAG GAAGGTGGTA GGTCAAGTGT
1081 GGCCACCATT GGGGAGACGA GAAAGAAGTG GGGCCCCACC AGATTGCACA ATGGGAACCT
1141 CAGCTGGCCC CTGAACAGAG GACTCAGTTG TCTCCACCCT ACACCGCTAT TCCCTGGAGC
1201 TCAGCCAGGC GCAGCCTTGG AAGGAGAAAG GGCTGGGGTT ACCTGGCTTG TCCCTCCTCA
1261 GGAAAGCCCC CTTCTCTCTC TGCCCCAGCT CCCAGCCTGG CCTCCTCCAG GCAGGCCCTA
1321 CTCTCTGCC CCAGCTCCGG CTTTCCCCAT GAGGTTTGTC CCAGGCATGA AGAAAGCATC
1381 CAGGGTGCCA ATGAGTGGGC CTAGGCCAGA GGCCCCCAG TCCCCAAGGG TACTGTTTTG
1441 GTGGCCTTTC AGAGGGTCAA GGAAGCCCTG CTTGGGGTAG AAGGGGCAGG AGCCCCACAT
1501 GTTGGGGGAG GAAATAAAGT GGAGTGTGCT GTGCTGAAAA AAAAAAAAAA AAAA



```

TAA Stop codon

AATAAA Consensus polyadenylation site

FIGURE 6

Primer	Sequence (5'-3')
T3	ATT AAC CCT CAC TAA AGG GA
T7	TAA TAC GAC TCA CTA TAG GG
SP6	ATT TAG GTG ACA CTA TAG

1	CTGTTCAGCAA	CCAGCAACAA	ATGGCCCATG	GGTGGTAGTG	GTGTCGTGGA	GGTCCCCTTC
			m g g s g v v e v p f			
	78836-FL-F					
61	CTGCTCTCCA	GCAAGTACGA	TGAGCCCAGC	CGCCAGGTCA	TCCTGGAGGC	TCTTGCGGAG
	l l s	s k y	d e p s	r q v	i l e	a l a e
121	TTTGAACGTT	CCACGTGCAT	CAGGTTTGTC	ACCTATCAGG	ACCAGAGAGA	CTTCATTTC
	f e r	s t c	i r f v	t y q	d q r	d f i s
181	ATCATCCCCA	TGTATGGGTG	CTTCTCGAGT	GTGGGGCGCA	GTGGAGGGAT	GCAGGTGGTC
	i i p	m y g	c f s s	v g r	s g g	m q v v
241	TCCCTGGCGC	CCACGTGTCT	CCAGAAGGGC	CGGGGCATTG	TCCTTCATGA	GCTCATGCAT
	s l a	p t c	l q k g	r g i	v l h	e l m h
301	GTGCTGGGCT	TCTGGCACGA	GCACACGCGG	GCCGACCGGG	ACCCTATAT	CCGTGTCAAC
	v l g	f w h	e h t r	a d r	d r y	i r v n
361	TGGAACGAGA	TCCTGCCAGG	CTTTGAAATC	AACTTCATCA	AGTCTCAGAG	CAGCAACATG
	w n e	i l p	g f e i	n f i	k s q	s s n m
421	CTGACGCCCT	ATGACTACTC	CTCTGTGATG	CACTATGGGA	GGCTCGCCTT	CAGCCGGCGT
	l t p	y d y	s s v m	h y g	r l a	f s r r
481	GGGCTGCCCA	CCATCACACC	ACTTTGGGCC	CCCAGTGTCC	ACATCGGCCA	GCGATGGAAC
	g l p	t i t	p l w a	p s v	h i g	q r w n
541	CTGAGTGCCT	CGGACATCAC	CCGGGTCCTC	AAACTCTACG	GCTGCAGCCC	AAGTGGCCCC
	l s a	s d i	t r v l	k l y	g c s	p s g p
601	AGGCCCCGTG	GGAGAGGGTC	CCATGCCCAC	AGCACTGGTA	GGAGCCCCGC	CCCGGCCTCC
	r p r	g r g	s h a h	s t g	r s p	a p a s
661	CTATCTCTGC	AGCGGCTTTT	GGAGGCACTG	TCGGCGGAAT	CCAGGAGCCC	CGACCCCAGT
	l s l	q r l	l e a l	s a e	s r s	p d p s
721	GGTTCCAGTG	CGGGAGGCCA	GCCCGTTCCT	GCAGGGCCTG	GGGAGAGCCC	ACATGGGTGG
	g s s	a g g	q p v p	a g p	g e s	p h g w
781	GAGTCCCCTG	CCCTGAAAAA	GCTCAGTGCA	GAGGCCCTCG	CAAGGCAGCC	TCAGACCCTA
	e s p	a l k	k l s a	e a s	a r q	p q t l
841	GCTTCCTCCC	CAAGATCAAG	GCCTGGAGCA	GGTGCCCCCG	GTGTTGCTCA	GGAGCAGTCC
	a s s	p r s	r p g a	g a p	g v a	q e q s
901	TGGCTGGCCG	GAGTGTCCAC	CAAGCCCACA	GTCCCATCTT	CAGAAGCAGG	AATCCAGCCA
	w l a	g v s	t k p t	v p s	s e a	g i q p
961	GTCCCTGTCC	AGGGAAGCCC	AGCTCTGCCA	GGGGGCTGTG	TACCTAGAAA	TCATTTCAAG
	v p v	q g s	p a l p	g g c	v p r	n h f k
1021	GGGATGTCCG	AAGATTAAGC	CTGTGGCT			
			g m s e d			
	78836-FL-R					

**FIGURE 8**

Query= INSP005a  
(336 letters)

Database: All non-redundant GenBank CDS  
translations+PDB+SwissProt+PIR+PRF  
1,247,039 sequences; 397,579,747 total letters

Searching.....done

Sequences producing significant alignments:			Score (bits)	E Value
ref	XP_141346.1	similar to hatching enzyme EHE7 [Anguilla japon...	416	e-115
dbj	BAB68518.1	hatching enzyme EHE13 [Anguilla japonica]	187	2e-46
dbj	BAB68515.1	hatching enzyme EHE7 [Anguilla japonica]	186	4e-46
dbj	BAB68516.1	hatching enzyme EHE10 [Anguilla japonica]	186	4e-46
dbj	BAB68513.1	hatching enzyme EHE4 [Anguilla japonica]	186	5e-46
dbj	BAB68517.1	hatching enzyme EHE12 [Anguilla japonica]	183	3e-45
dbj	BAB68514.1	hatching enzyme EHE6 [Anguilla japonica]	183	3e-45
dbj	BAB68519.1	hatching enzyme EHE14 [Anguilla japonica]	182	7e-45
pir	C48826	high choriolytic hatching proteinase (EC 3.4.24.-) H...	171	1e-41
dbj	BAA12146.1	choriolysin H [Oryzias latipes]	171	2e-41

**Top alignment to known metalloproteinase:**

>dbj|BAB68518.1| hatching enzyme EHE13 [Anguilla japonica]  
Length = 271

Score = 187 bits (475), Expect = 2e-46  
Identities = 93/183 (50%), Positives = 124/183 (66%), Gaps = 3/183 (1%)

Query: 5 GVVEVPFLLSSKYDEPSRQVILEALAEFERSTCIRFVTYQDQDFISIIIPMYGCFSSVGR 64  
G+VEVP+ +SS++ ++ I A+ F TCIRFV QRDFISI GC+S +GR  
Sbjct: 91 GLVEVPYTVSSEFSYHKKRIENAMETFNTETCIRFVPRSSQRDFISIESRDGCYSYLGR 150

Query: 65 SGGMQVVS LAPT-CLQKGRGIVLHELMHVLGFWHEHTRADRDYIRVNWNEILPGFEINF 123  
+GG QVVSLA C+ GI+ HEL H LGF+HEHTR+DRD Y+++NW + P NF  
Sbjct: 151 TGCKQVVS LARYGCVY--HGIIQHELNHALGFYHEHTRSDRDEYVKINWENVAPHTIYNF 208

Query: 124 IKSQSSNMLTPYDYSSVMHYGR LAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKL 183  
+ ++N+ TPYDY+S+MHYGR AFS G+ TITP+ P+ IGQR ++S DI R+ KL  
Sbjct: 209 QEQDTNNLNTPYDYTSIMHYGRTAFSTNGMDTITPVPNPNQSIGQRRSMKGDILRINKL 268

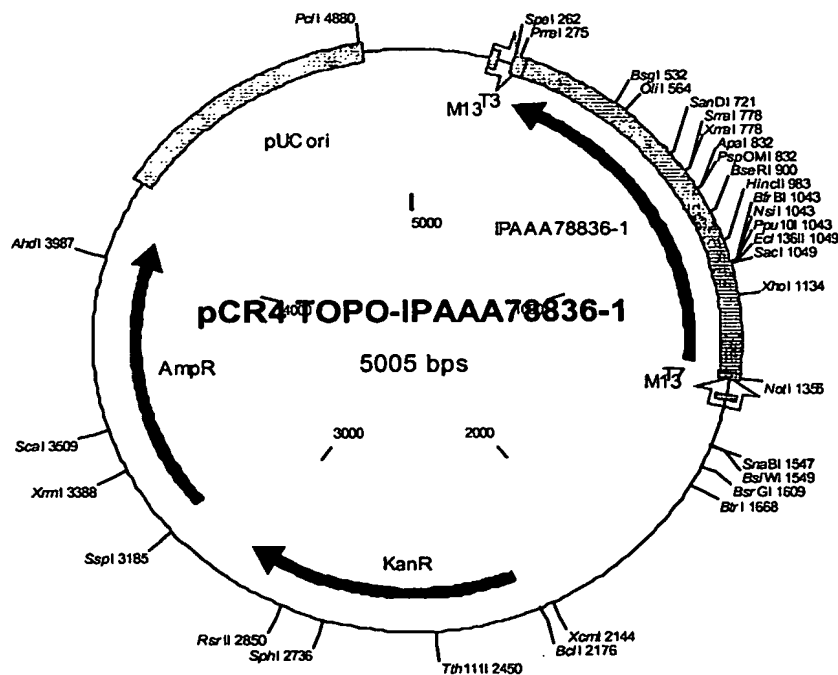
Query: 184 YGC 186  
Y C  
Sbjct: 269 YSC 271



Molecule: pCR4 TOPO-IPAAA78836-1, 5005 bps DNA Circular  
File Name: 13164.cm5, dated 24 Oct 2002

Description: Ligation of inverted 78836\_F2/R8 PCR product into pCR4-TOPO linear vector\*

Type	Start	End	Name	Description
REGION	205	221	M13	rev priming site
MARKER	243		T3	
REGION	262	294		Polylinker'
REGION	294	294		TOPO cloning site'
GENE	1315	308	C IPAAA78836-1	
REGION	1342	295	C	Inserted PCR product
REGION	1343	1360		'Polylinker
REGION	1343	1343		'TOPO cloning site
MARKER	1395		C T7	
REGION	1403	1418	M13	
GENE	2207	3001	KanR	
GENE	3205	4065	AmpR	
REGION	4210	4883	pUC ori	



10/18

FIGURE 10

78836-FL2-F

1 TTCTACCATG GAGGGTGTAG GGGGTCTCTG GCCTTGGGTG CTGGGTCTGC TCTCCTTGCC  
                   m e g v → g g l w p w v l g l l s l

61 AGGTGTGATC CTAGGAGCGC CCCTGGCCTC CAGCTGCGCA GGAGCCTGTG GTACCAGCTT  
     p g v i l g a p l a s s c a g a c g t s

121 CCCAGATGGC CTCACCCCTG AGGGAACCCA GGCCTCCGGG GACAAGGACA TTCCTGCAAT  
     f p d g l t p e g t q a s g d k d i p a

181 TAACCAAGGG CTCATCCTGG AAGAAACCCC AGAGAGCAGC TTCCTCATCG AGGGGGACAT  
     i n q g l i l e e t p e s s f l i e g d

241 CATCCG GCCG AGTCCCTTCC GACTGCTGTC AGCAACCAGC AACAAATGGC CCATGGGTGG  
     i i r p s p f r l l s a t s n k w p m g

301 TAGTGGTGTC GTGGAGGTCC CCTTCTGCT CTCCAGCAAG TACGATGAGC CCAGCCGCCA  
     g s g v v e v p f l l s s k y d e p s r

361 GGTATCCTTG GAGGCTCTTG CGGAGTTTGA ACGTTCCACG TGCATCAGGT TTGTACCTA  
     q v i l e a l a e f e r s t c i r f v t

421 TCAGGACCAG AGAGACTTCA TTTCCATCAT CCCCATGTAT GGGTGCTTCT CGAGTG TGGG  
     y q d q r d f i s i i p m y g c f s s v

481 GCGCAGTGGA GGGATGCAGG TGGTCTCCCT GGC GCCCAG TGTCTCCAGA AGGGCCGGGG  
     g r s g g m q v v s l a p t c l q k g r

541 CATTGTCTTT CATGAGCTCA TGCATGTGCT GGGCTTCTGG CACGAGCACA CGCGGGCCGA  
     g i v l h e l m h v l g f w h e h t r a

601 CCGGGACCGC TATATCCGTG TCAACTGGAA CGAGATCCTG CCAGGCTTTG AAATCAACTT  
     d r d r y i r v n w n e i l p g f e i n

661 CATCAAGTCT CGGAGCAGCA ACATGCTGAC GCCCTATGAC TACTCCTCTG TGATGCACTA  
     f i k s r s s n m l t p y d y s s v m h

721 TGGGAGGCTC GCCTTCAGCC GCGTGGGCT GCCCACCATC ACACCACTTT GGGCCCCCAG  
     y g r l a f s r r g l p t i t p l w a p

781 TGTCCACATC GGCCAGCGAT GGAACCTGAG TGCCTCGGAC ATCACCCGGG TCCTCAAACCT  
     s v h i g q r w n l s a s d i t r v l k

841 CTACGGCTGC AGCCCCAAGTG GCCCCAGGCC CCGTGGGAGA GGGTCCCATG CCCACAGCAC  
     l y g c s p s g p r p r g r g s h a h s

901 TGGTAGGAGC CCCGCTCCGG CCTCCCTATC TCTGCAGCGG CTTTTGGAGG CACTGTCCGC  
     t g r s p a p a s l s l q r l l e a l s

961 GGAATCCAGG AGCCCCGACC CCAGTGGTTC CAGTGC GGGA GGCCAGCCCG TTCTGCAGG  
     a e s r s p d p s g s s a g g q p v p a

1021 GCCTGGGGAG AGCCCACATG GGTGGGAGTC CCCTGCCCTG AAAAGCTCA GTGCAGAGGC  
     g p g e s p h g w e s p a l k k l s a e

11/18

1081 CTCGGCAAGG CAGCCTCAGA CCCTAGCTTC CTCCCAAGA TCAAGGCCTG GAGCAGGTGC  
a s a r q p q t l a s s p r s r p g a g

1141 CCCC GG TGT T GCTCAGGAGC AGTCCTGGCT GGCCGGAGTG TCCACCAAGC CCACAGTCCC  
a p g v a q e q s w l a g v s t k p t v

1201 ATCTTCAGAA GCAGGAATCC AGCCAGTCCC TGTCCAGGGA AGCCCAGCTC TGCCAGGGGG  
p s s e a g i q p v p v q g s p a l p g

1261 CTGTGTACCT AGAAATCATT TCAAGGGGAT GTCCGAAGAT TAAGCCTGTG GCT  
g c v p r n h f k g m s e d

78836-FL-R

12/18

**FIGURE 11**

Query= INSP005b

(431 letters)

Database: All non-redundant GenBank CDS

translations+PDB+SwissProt+PIR+PRF

1,247,039 sequences; 397,579,747 total letters

Searching.....done

	Score	E
Sequences producing significant alignments:	(bits)	Value
ref XP_141346.1  similar to hatching enzyme EHE7 [Anguilla japon...	540	e-152
dbj BAB68513.1  hatching enzyme EHE4 [Anguilla japonica]	198	1e-49
dbj BAB68518.1  hatching enzyme EHE13 [Anguilla japonica]	198	1e-49
dbj BAB68516.1  hatching enzyme EHE10 [Anguilla japonica]	197	3e-49
dbj BAB68515.1  hatching enzyme EHE7 [Anguilla japonica]	196	4e-49
dbj BAB68514.1  hatching enzyme EHE6 [Anguilla japonica]	196	7e-49
dbj BAB68517.1  hatching enzyme EHE12 [Anguilla japonica]	194	3e-48
dbj BAB68519.1  hatching enzyme EHE14 [Anguilla japonica]	191	1e-47
pir C48826 high choriolytic hatching proteinase (EC 3.4.24.-) H...	187	3e-46
dbj BAA12146.1  choriolysin H [Oryzias latipes]	186	4e-46

**Top alignment to known metalloproteinase:**

&gt;dbj|BAB68518.1| hatching enzyme EHE13 [Anguilla japonica]

Length = 271

Score = 198 bits (503), Expect = 1e-49

Identities = 103/233 (44%), Positives = 144/233 (61%), Gaps = 5/233 (2%)

Query: 52 DKDIPAINQGLILEETPESSFLIEGDIIRPSPFRLLSATSNNK--WPMGGSGVVEVPFLLS 109

D D I ++ S L+EGD++ + ++ +N+ W G+VEVP+ +S

Sbjct: 41 DPDDLITARILQSNNGSSEILMEGDMVSNTRNAINCWNQCLWRKSSDGLVEVPYTVS 100

Query: 110 SKYDEPSRQVILEALAEFERSTCIRFVTYQDQRDFISIIIPMYGCFSSVGRSGGMQVVSLA 169

S++ ++ I A+ F TCIRFV QRD FISI GC+S +GR+GG QVVSLA

Sbjct: 101 SEFSYYHKKRIENAMETFNTETCIRFVPRSSQDRDFISIESRDGCYSYLGRTGGKQVVSLA 160

Query: 170 PT-CLQKGRGIVLHELMHVLGFWHEHTRADRDYIRVNNWNEILPGFEINFIKSRSSNMLT 228

C+ GI+ HEL H LGF+HEHTR+DRD Y+++NW + P NF + ++N+ T

Sbjct: 161 RYGCYV--HGIIQHELNHALGFYHEHTRSDRDEYVKINWENVAPHTIYNFQEQDTMNLNT 218

Query: 229 PYDYSSVMHYGRLAFSRRGLPTITPLWAPSVHIGQRWNLSASDITRVLKLYGC 281

PYDY+S+MHYGR AFS G+ TITP+ P+ IGQR ++S DI R+ KLY C

Sbjct: 219 PYDYTSIMHYGRTAFSTNGMDTITPVPNPNQSIGQRRSMKGDILRINKLYSC.

13/18

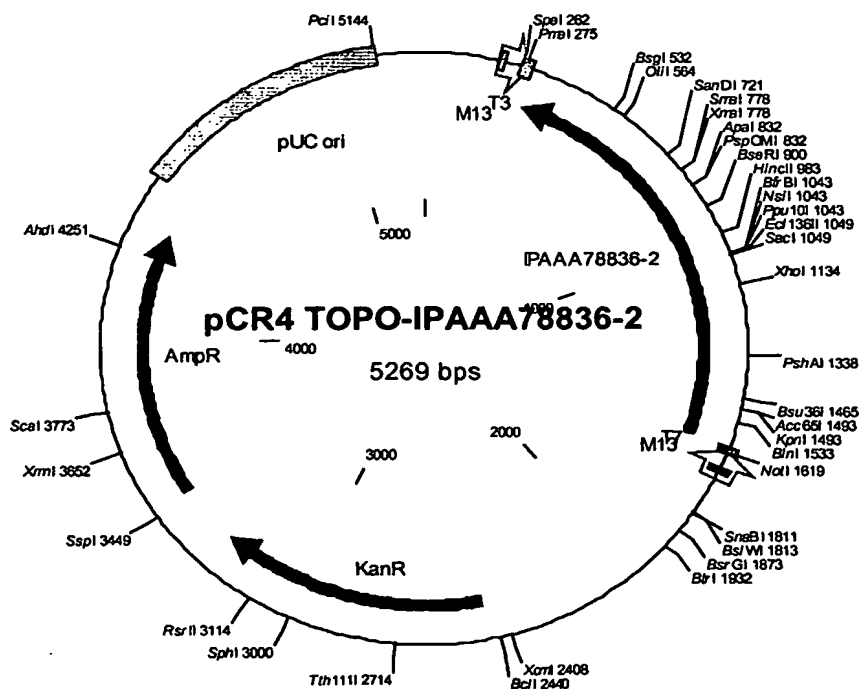
**FIGURE 12**

Molecule: pCR4 TOPO-IPAAA78836-2, 5269 bps DNA Circular  
 File Name: 13296.cm5, dated 24 Oct 2002

Description: Ligation of inverted IPAAA78836v2 into pCR4-TOPO linear vector\*

**Molecule Features:**

Type	Start	End	Name	Description
REGION	205	221	M13	rev priming site
MARKER	243		T3	
REGION	262	294		Polylinker'
REGION	294	294		TOPO cloning site'
GENE	1600	307	C IPAAA78836-2	
REGION	1607	1624		'Polylinker
REGION	1607	1607		'TOPO cloning site
MARKER	1659		C T7	
REGION	1667	1682	M13	
GENE	2471	3265	KanR	
GENE	3469	4329	AmpR	
REGION	4474	5147	pUC ori	



[illegible]

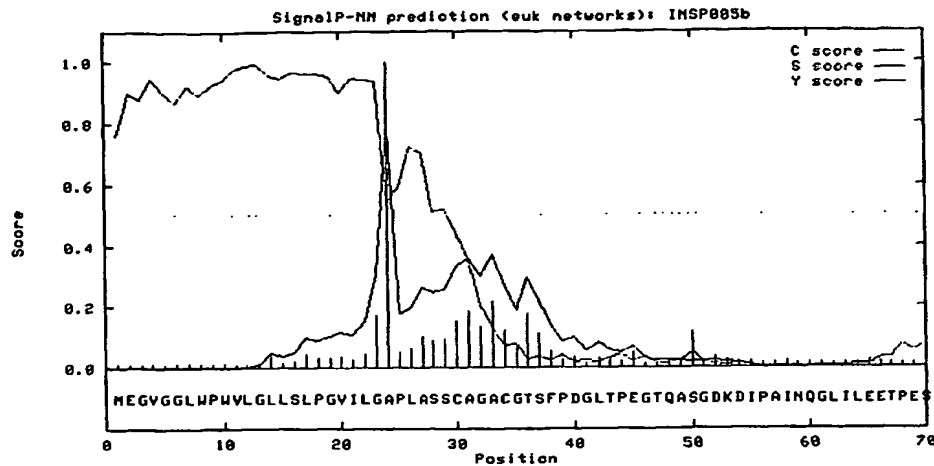


16/18

FIGURE 14

&gt;INSP005b

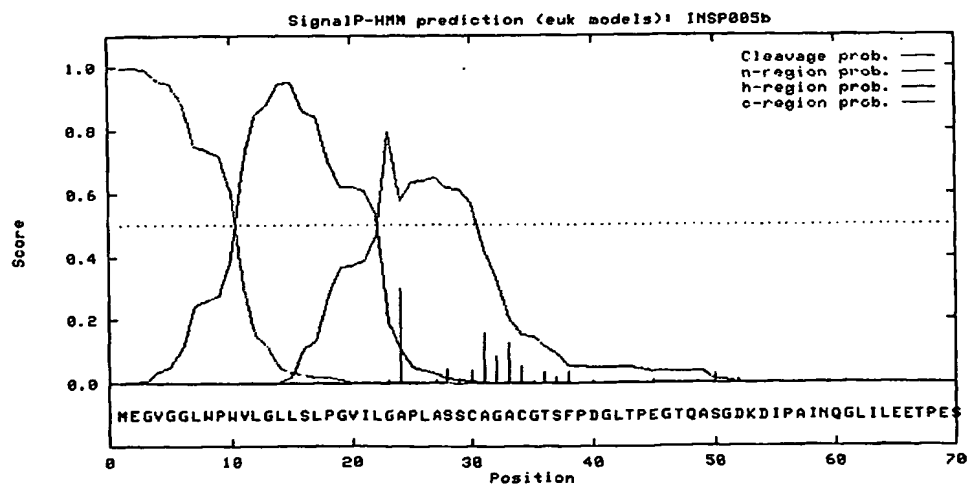
SignalP-NN result:



# data

```
>INSP005b                                length = 70
# Measure  Position  Value  Cutoff  signal peptide?
max. C      24       1.000  0.33   YES
max. Y      24       0.789  0.32   YES
max. S      13       0.991  0.82   YES
mean S      1-23     0.929  0.47   YES
# Most likely cleavage site between pos. 23 and 24: ILG-AP
```

SignalP-HMM result:



# data

```
>INSP005b
Prediction: Signal peptide
Signal peptide probability: 0.996
Signal anchor probability: 0.003
Max cleavage site probability: 0.302 between pos. 23 and 24
```



FIGURE 15A

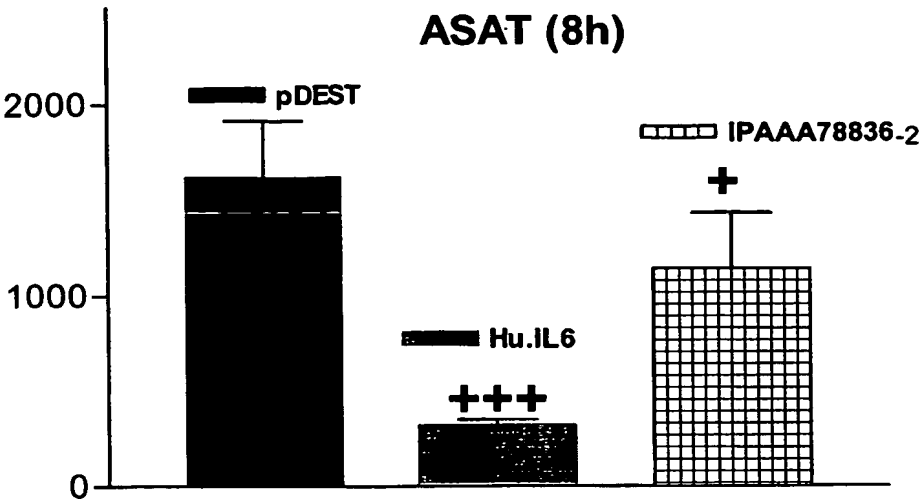
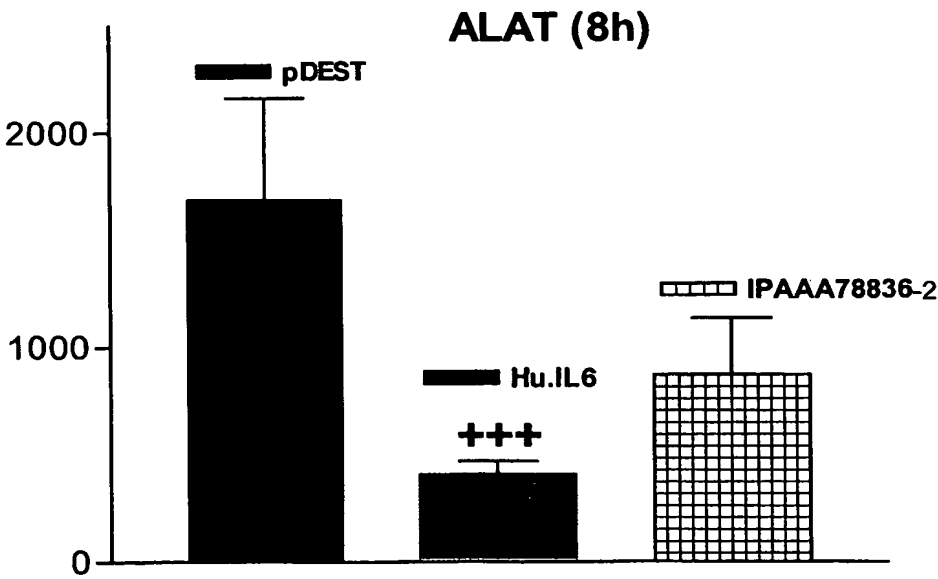


FIGURE 15B



18/18

FIGURE 16A

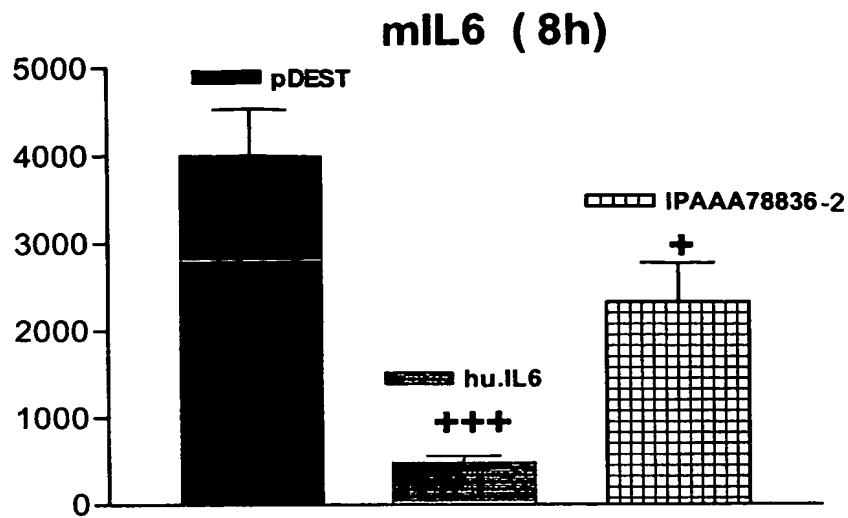


FIGURE 16B

